St. Louis University
Medical Center



Grant NAGW-2708:

Final Technical Report:

The funds from this grant helped test hardware for the Spacelab-Japanese Bone Cell Research Experiment. The major limitation of the experiment was that we were only able to have two chambers for the flight experiment. One would serve as a Control chamber, the other as a PTHrP-treated chamber. This meant that no statistical evaluation of the data could be conducted. Media were collected at several times to give baseline data and for PTHrP treatment for stimulation of media collagenase (13 h) and TIMPs (52 h). As well, microphotography of the cells was conducted at multiple times. At the end of the experiment the cells were lysed for later protein assay. The limitations of the chamber, numbers of chambers, flight and preservation of samples meant we could not carry out investigation of collagen synthesis (usually done by [$^3$H]-proline labelling) nor the latter two aims of the grant. These may be able to be accomplished in future flights with different hardware and greater numbers of samples.

During the SL-J flight, our experience was that photomicroscopy and media exchanges generally went well. The major problem we encountered was the loss of dividing cells during the first media exchange due to misinterpretation by the crew of this procedure. The communication link was satisfactory. However, even though we were aware that the Japanese astronaut was performing the first media exchange incorrectly, my team was prevented from communicating with him to tell him to change what he was doing. This one step affected all of our science since our cells never reached confluence.

Down link communication indicated sizeable cell loss in flight chambers. We conducted parallel ground controls which did lose a majority of cells during first media exchange due both to duplication of the crew's activities and inferior chambers in which cell adhesion was not good. Our best chambers were used for flight.

Post-flight, we repeated the ground control portion of the experiment protocol using the flight chambers and had better cell growth. Repetition of the ground control experiment has helped to confirm slight differences in morphology of the flight cells.

We assayed the flight and ground control samples for collagenase by ELISA. Due to sizeable loss of dividing cells during flight and in the parallel ground controls, the results were below the limit of detection for the standard curve. However, we were able to concentrate the media before assaying and the results are shown in Table 1.

We were unable to measure rat TIMPs by an ELISA since the affinities of the antisera we obtained were insufficient and also cross-reacted with bovine TIMPs (abundant in fetal bovine serum, FBS). The alternative method would be to measure the TIMPs by a functional assay but this was also not possible since the BCR media samples contained 10% FBS. This problem would be exacerbated by

concentration of the media 50-fold.

In conclusion, there appear to be some possible morphological changes in the cells under microgravity but we are unable to determine whether these are due to microgravity or the conditions of lift-off and/or vibration.

Table 1. Collagenase Concentrations produced by UMR Cells in SL-J Chambers

| | MMP-1 Concentration ($\mu$g/chamber) |
|---|---|
| Ground Control | |
| MET 0/09:50, Test X-change #1. | 573 |
| MET 0/10:00, Control X-change #1. | 1,276 |
| MET 2/23:20, Test X-change #2. | 321 |
| MET 2/23:30, Control X-change #2. | 211 |
| MET 3/12:27, Test X-change #3. | 606 |
| MET 3/12:35, Control X-change #3. | 647 |
| MET 6/13:06, Test X-change #4. | 352 |
| MET 6/13:13, Control X-change #4. | 352 |
| Flight Samples | |
| MET 0/09:50, Test X-change #1 | 855 |
| MET 0/10:02, Control X-change #1 | 769 |
| MET 2/23:35, Test X-change #2 | 3,472 |
| MET 2/23:50, Control X-change #2 | 4,900 |
| MET 3/13:47, Test X-change #3 | 8,134 |
| MET 3/13:58, Control X-change #3 | 10,422 |
| MET 6/13:27, Test X-change #4 | 6,254 |
| MET 6/13:33, Control X-change #4 | 9,597 |

UMR cells were cultured in flight SL-J chambers (22 ml medium/chamber) and then had the media exchanges indicated. At the second and third media exchanges the test chambers received PTHrP ($10^{-7}$ M). The media were concentrated 50-fold for these data since straight media gave MMP-1 values which were at the bottom of the ELISA standard curve. However, concentration may cause FBS interference in the assay. Notwithstanding, all samples had the same amount of FBS so the contribution should be the same.